

Evaluation of α_1 -Antitrypsine and Reduced Glutathione in Iraqi Patients of Diabetes Mellitus Type II.

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Abstract

In order to investigate the levels of reduced glutathione GSH and α_1 -antitrypsine in the sera of 20 type 2 diabetic patients and 10 healthy subjects, were enrolled in this study. A significant reduction in GSH level was found in the patient group compared with control.

On the other hand a significant elevation in α_1 -antitrypsine in patient compared with control was observed.

Correlation between α_1 -antitrypsine and reduced glutathion was found to be positive (+Ve) for diabetes mellitus type2 patients and negative (-Ve) for healthy control with r values 0.257 and - 0.339 respectively.

In conclusion the depletion of GSH as antioxidant defense insured higher free radical generation in diabetic patients which is conformed by the high α_1 -antitrypsine level in the sera.

Key words: α_1 -Antitrypsine , Glutathione , Diabetes Mellitus Type 2

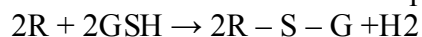
Introduction

Diabetes mellitus DM is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized by the body tissue, producing hyperglycemia. Some patients may develop life threatening conditions like keto-acidosis and coma.[1]

Over the last decade, there has been a significant interest in oxidative stress and its role in the development of complications in diabetic patients.[2]

Glutathione (GSH) (γ -glutamyl-cysteinyl glycine) is a tripeptide consisting of glutamic acid, cysteine and glycine.

A number of potentially toxic electrophilic xenobiotics are conjugated to the nucleophilic GSH in reactions that can be represented as follows:



Where R= an electrophilic xenobiotic

If the potentially toxic xenobiotics were not conjugated to GSH they would be free to combine covalently with DNA, RNA or cell protein and could thus lead to serious cell damage.[2]

GSH has other important functions in human cells apart from its role in xenobiotic metabolism. It participates in the decomposition of potentially toxic hydrogen peroxide in the reaction catalyzed by glutathione peroxidase, it is an important intracellular reductant helping to maintain essential SH groups of enzymes in their reduced state. A metabolic cycle involving GSH as a carrier has been implicated in the transport of certain amino acid across membranes in the kidney.[3]

Alpha 1-antitrypsine is among positive acute phase proteins (Apps) which is a major pathophysiologic phenomenon that accompanies inflammation either acute or chronic[4], the release of inflammatory molecules (cytokines) changes the level of Apps as well as a number of behavioral, physiologic, biochemical and nutritional changes are induced.[5]

The aim of the present study is to evaluate the levels of GSH as a major endogenous antioxidant and α_1 -antitrypsine as a reactant modulated during inflammatory diseases in the sera of type 2 DM Iraqi patients and to correlate both parameters.

Experimental

Subjects:

The study group comprised 20 patients from both sexes with type 2 DM diagnosed by physicians at AL-Kadhyamia teaching hospital in addition to ten healthy control matching the age.

Blood samples:

About five milliliters of venous blood were collected from each subject in the study after a 12-hour fast.

The blood samples were collected into heparin tubes, left at room temperature for 15 min then centrifuged at 3000 rpm for 15 min. Serum was separated and aliquoted for subsequent measurement of GSH and α_1 -antitrypsine..

Laboratory methods:

Determination of glutathion concentration GSH.

Reduced glutathione was determined according to the method of Ellman[6], based on the reaction of aliphatic thiol compounds with 5,5-dithiobis (2-nitrobenzoic acid)(DTNB) at pH8.

The absorbance of the yellow chromagen was measured at 412 nm and is directly proportional to GSH concentration. So one mole of the thiol produces one mole of p-nitro thiophenol anion which is highly coloured ($\epsilon=13600 \text{ M}^{-1} \text{ cm}^{-1}$)

Determination of α_1 -antitrypsine:

Alpha one antitrypsine was measured in the sera of patients and healthy control groups using a ready kit from Bindarid UK RNO 34.3.

The method involves antigen diffusing radially from a cylindrical well through an agarose gel containing an appropriate mono-specific antibody.

Antibody- Antigen- complexes are formed which, under the right conditions, will form a precipitin ring. The ring size will increase until equilibrium is reached between the formation and breakdown of these complexes, this point is termed completion. At this stage, a linear relationship exists between the square of this ring diameter and the antigen concentration.

By measuring the ring diameters produced by a number of samples of known concentration, a calibration curve may be constructed. The concentration of the antigen in an unknown sample may then be determined by measuring the ring diameter produced by that sample and reading off the calibration curve[7].

Statistical analysis:

Data are expressed as mean standard deviation of (mean \pm S.D.). Statistical significance was determined by unpaired student's, t-test. One way of analysis of variance (ANOVA) is followed by Pearson's correlation (r). P values equal or lower than 0.05 were considered statistically significant.

Results and Discussion

Reduced glutathione GSH:

Table 1 shows the level mean \pm SD of GSH in the serum of type 2 DM patient and healthy control, a significant decrease in GSH level in the serum of type 2 DM patient compared with the control was found with values 1.092 ± 0.204 M and 3.4 ± 0.529 M respectively. These results are in agreement with recent study reported that a significant decrease in GSH level in patient with type 2 DM [8].

Reduced glutathione is physiological free radical scavengers. Thus glutathione plays a central role in antioxidant defense [9]. In hyperglycemic condition, glucose is preferentially used in the polyol pathway that consumes NADPH which is necessary for GSH regeneration by the glutathione reductase enzyme [10].

Many reports showed that diabetic humans have shown increased lipid peroxidation and decreased levels of reduced glutathione and these results suggest that the increase in lipid peroxidation, and the decline in antioxidant defences may appear early in type 2 non-insulin dependent diabetes mellitus patients, before the development of secondary complications [11,12].

α_1 -antitrypsine:

A significant increase in α_1 -antitrypsine levels was found between DM patients which was 1877.11 ± 222.889 mg/L compared with healthy control which was 950.00 ± 104.88 mg/L

The results of the present study is in agreement with reported data claimed an increase in the levels of acute-phase protein including α_1 -antitrypsine in adult diabetes (principally type (2)) [13], also results proposed a novel biological function for α_1 -antitrypsine and suggest that it may represent an effective candidate for attempts seeking to prevent or reverse type 1 diabetes [14]. On the other, a study is on α_1 -antitrypsine in diabetes in both.

Type I and II diabetes mellitus are associated with reduced α_1 -antitrypsine as serine protease inhibitory capacity of plasma [15].

Fig (1) shows correlation relation between α_1 -antitrypsine and reduced glutathione for DM type II patients and healthy control. The correlation was found to be (+Ve) for DMII patients and (-Ve) for healthy control with r values 0.257 and -0.339 respectively.

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Table(1): Shows the level, mean and SD of reduced glutathione in sera of type 2 DM patient and healthy control

P value	SD	mean	N	GSH M
	± 0.204	1.092	20	Patient
	± 0.529	3.400	10	Control

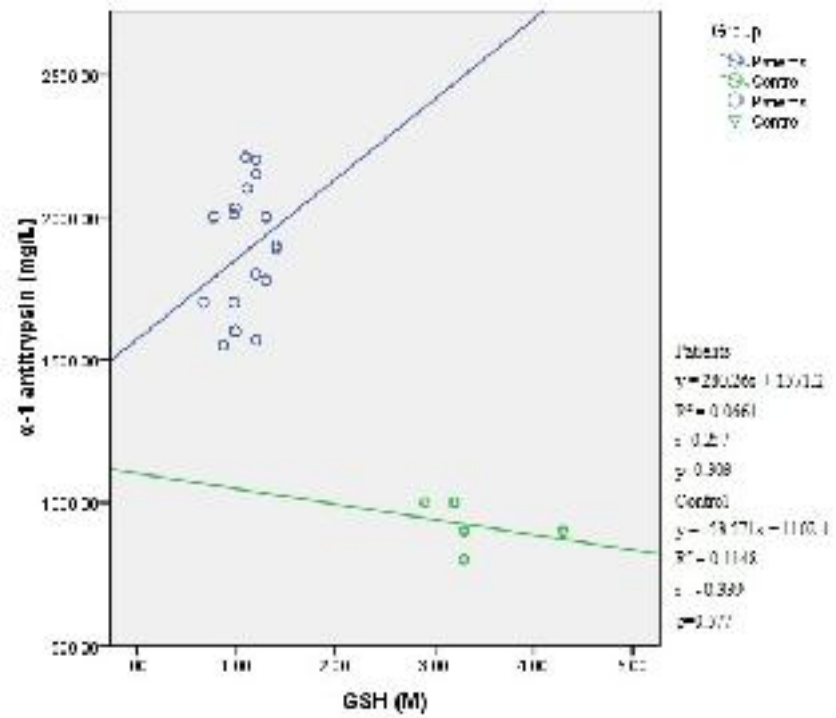
S: significant

Table(2): Shows the level, mean and SD of α_1 –antitrypsine in the sera of type 2 DM patient and healthy control

P value	SD	mean	N	α_1 –antitrypsin mg/L
	± 222.889	1877.111	20	Patient
	± 104.881	950.000	10	Control

S: significant

Fig.(1): The correlation relation between α_1 –antitrypsin and reduced glutathione for DM 2 patints and healthy control.



تقدير الكلوتاثيون المختزل α_1 -antitrypsin GSH للمرضى العراقيين المصابين بداء السكري من النوع الثاني

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الخلاصة

لدراسة مستويات (الكلوتاثيون المختزل GSH والمضاد للتربين من بوصفهم α_1 -antitrypsine في مصل عشرين مصاباً بداء السكري من النوع الثاني فضلا عن عشرة من الاصحاء بوصفهم مجموعة سيطرة ، اذ وجد في هذه الدراسة نقصان مميز لمستوى الكلوتاثيون المختزل GSH لمرضى داء السكري من النوع الثاني مقارنة مع الاصحاء. وفي الجانب الآخر لوحظت زيادة مميزة لمستوى α_1 -antitrypsine لأولئك المرضى بالمقارنة مع أنظارهم من الاصحاء. لوحظت العلاقة بين α_1 -antitrypsine والكلوتاثيون المختزل لمرضى داء السكري من النوع الثاني (+Ve) و (- Ve) للاصحاء وكانت قيمة (r) مساوية لـ (0,257) و (-0,337) على التوالي. وقد لوحظ من النتائج ان استهلاك الكلوتاثيون المختزل GSH الحامي ضد الاكسدة يؤكد تخليق الجذور الحرة بنسبة عالية لدى المرضى الذي يكون مطابقاً للمستوى العالي α_1 -antitrypsine في مصل المصابين .

الكلمات المفتاحية: مضاد التربسين نوع α_1 ، الكلوتاثيون ، داء السكري النوع الثاني